Serological Evidence that *Yersinia enterocolitica* Lipopolysaccharide Produced during Growth *in vivo* Resembles that Produced during Growth *in vitro* at 25 °C

By YOSHIHIRO KAWAOKA,* KOICHI OTSUKI AND
MISAO TSUBOKURA

Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori-shi,
Tottori 680, Japan

(Received 30 November 1982; revised 25 March 1983)

Partial smooth-rough transition was observed in 30 strains of *Yersinia enterocolitica* O:3 cultivated at 37 °C. Immunodiffusion and haemagglutination inhibition tests with eight patients' sera demonstrated that the immunogenicity of the lipopolysaccharide of *Yersinia enterocolitica* *in vivo* was similar to that of the bacteria grown *in vitro* at 25 °C.

INTRODUCTION

Yersinia enterocolitica cells grown at 25 °C are quite different in their biochemical and biological characteristics from those grown at 37 °C. For example, adherence to mammalian epithelial cell lines (Okamoto *et al.*, 1980), motility (Schleifstein & Coleman, 1939), indirect haemolysin (Tsubokura *et al.*, 1979) and enterotoxin (Pai & Mors, 1978) production, and bacteriophage sensitivity (Mollaret & Nicolle, 1965) are observed with bacteria grown at 25 °C but not with those grown at 37 °C. It is not known, however, whether the bacteria grown *in vivo* resemble more closely those grown *in vitro* at 25 °C or at 37 °C.

We have shown, in the preceding paper, that partial smooth-rough transition occurs in *Y. enterocolitica* grown at 37 °C (Kawaoka *et al.*, 1983). A rabbit antiserum prepared against 25 °C-grown bacteria contained antibodies directed mainly against the O-antigenic polysaccharide (O-PS) portion and to a smaller extent against the R-core of lipopolysaccharide (LPS). By contrast, a rabbit antiserum against 37 °C-grown bacteria contained antibodies directed mainly against the R-core of LPS. From these results, we considered that the specificity of the antibody in sera from patients with clinical infection would indicate whether *Y. enterocolitica* organisms grown *in vivo* are similar in immunogenicity to the bacteria grown *in vitro* at 25 °C or at 37 °C.

METHODS

Bacterial strains. *Yersinia enterocolitica* O:3 (30 strains) isolated from various animals (humans, pigs, dogs, cats, monkeys, and rats) in various countries were received from Drs D. P. Falcão (Brazil), H. Fukushima (Japan), W. Knapp (West Germany), T. Maruyama (Japan), H. H. Mollaret (France), G. Wauters (Belgium), S. Winblad (Sweden).

Preparations of lipopolysaccharide (LPS) and O-antigenic polysaccharide (O-PS). LPS was prepared from *Y. enterocolitica* strain Ye 3827 (serovar O:3) grown at 25 °C (25 °C-LPS) or 37 °C (37 °C-LPS) by the method of Westphal & Jann (1965). O-PS was prepared by hydrolysis of LPS with 1% (v/v) acetic acid as previously described (Davies *et al.*, 1955).

Antisera. Antisera to smooth cells, Ye 3827, grown at 25 °C (25 °C-bacteria) or at 37 °C (37 °C-bacteria) and to rough cells, Ye 3827 IV-3, grown at 25 °C (R-bacteria) were prepared with heat-killed cells.

Human sera. Human sera were collected about 3 weeks after the onset of symptoms from eight patients (12–15 years old) with *Y. enterocolitica* infection; these samples were kindly supplied by Dr M. Inoue (Okayama Prefectural Research Institute of Environment and Public Health, Okayama, Japan).

Immunological methods. Double immunodiffusion tests in 1% (w/v) agarose gel were performed as described previously (Kawaoka *et al.*, 1979). Each antigen was dissolved in phosphate-buffered saline containing 0.1% sodium taurocholate.

Fig. 1. Immunodiffusion in 1% (w/v) agarose gel of the antisera against Ye 3827 cells grown at 25 °C (1A) and 37 °C (2A) and the patient's serum no. 4344 (P) with LPS prepared from Ye 3827 cells grown at 25 °C (1) and 37 °C (2). The results shown in (a) were from experiments described in the accompanying paper (Kawaoka *et al.*, 1983).

Passive haemagglutination inhibition (HI) tests were performed according to Lindberg & Hellerqvist (1980) with sheep erythrocytes sensitized with LPS. Agglutination was read after incubation for 1 h at 37 °C and after an additional 18 h in the cold.

RESULTS

The partial smooth-rough transition occurring in *Y. enterocolitica* strain Ye 3827 grown at 37 °C has been shown in the preceding paper (Kawaoka *et al.*, 1983). To demonstrate how common this phenomenon was among *Y. enterocolitica* O:3 strains, we examined 30 strains from different origins for the reactivity to rabbit antiserum raised against 25 °C-bacteria and against R-bacteria. All strains grown either at 25 °C or at 37 °C were agglutinable with a 1:10 dilution of the antiserum to 25 °C-bacteria. On the other hand, only the 37 °C-grown bacteria were agglutinable with a 1:10 dilution of the antiserum to R-bacteria. Thus, partial smooth-rough transition commonly occurred in *Y. enterocolitica* O:3 strains irrespective of species or country of origin.

Serum from a patient (no. 4344) showed the same results as those obtained with the antiserum to 25 °C-bacteria in the immunodiffusion test (Fig. 1). The patient's serum formed two precipitation lines against 25 °C-LPS; one was specific to 25 °C-LPS and the other was common to both 25 °C- and 37 °C-LPS. The same results were obtained with sera from seven other patients.

In the HI tests, the patients' sera gave results similar to those obtained with the antiserum against 25 °C-bacteria (Table 1). O-PS from either 25 °C- or 37 °C-LPS inhibited the reaction between the patients' sera or the antiserum to 25 °C-bacteria and the erythrocytes coated with 25 °C-LPS, though amounts required for inhibition differed among the individual patients' sera. The data indicate that a major component of the reaction between patients' sera and 25 °C-LPS involved O-PS and its corresponding antibody. Both 25 °C- and 37 °C-LPS inhibited all the reactions.

DISCUSSION

When 30 strains of *Y. enterocolitica* O:3 were cultivated at 37 °C, partial smooth-rough transition was observed in each of them. The generality of the phenomenon among *Y. enterocolitica* O:3 strains made it possible to investigate the immunogenicity of the *in vivo* grown bacteria using sera from patients.

Table 1. *Passive HI tests with erythrocytes coated with LPS prepared from Ye 3827 cells grown at 25 °C*

LPS and O-PS were examined for potency of inhibition of antigenic binding in an HI system using sheep erythrocytes sensitized with LPS.

Inhibitor	Amount of inhibitor necessary for complete inhibition of haemagglutination (ng)									
	Antiserum against:		Patients' sera (no.):							
	Ye 3827 (25 °C)	Ye 3827 (37 °C)	4343	4344	4345	4346	4347	4348	4367	4389
Ye 3827 (25 °C)										
LPS	6	390	195	12	97	97	12	6	12	48
O-PS	1560	—	1560	390	6250	1560	780	780	390	3125
Ye 3827 (37 °C)										
LPS	12	390	195	24	195	97	24	12	12	48
O-PS	1560	—	1560	390	6250	1560	1560	780	780	1560

—, > 12 500 ng.

It was demonstrated by immunodiffusion and HI tests with eight patients' sera that immunogenicity of LPS of *Y. enterocolitica* cells *in vivo* is similar to that of the bacteria grown *in vitro* at 25 °C. However, the difference of immunogenicity between the bacteria grown *in vivo* and those grown *in vitro* at 37 °C may not be attributed directly to that of LPS structure. The poor anti-O response in rabbits immunized with 37 °C-bacteria can not be explained by the only moderately reduced amount of O-PS in such organisms (Kawaoka *et al.*, 1983). Therefore, it was proposed that the difference of immunogenicity might at least partially be due to other cell-surface properties, such as covering up of LPS by capsular-like material in the bacteria grown *in vitro* at 37 °C.

There is no previous evidence for expression *in vivo* of the several biological activities that characterize the growth temperature dependency of *Y. enterocolitica*. The present findings are the first to show that bacteria *in vivo* correspond in surface antigenic properties to organisms grown *in vitro* at 25 °C.

We thank Drs D. P. Falcão, H. Fukushima, W. Knapp, T. Maruyama, H. H. Mollaret, G. Wauters, S. Winblad for providing *Y. enterocolitica* O:3 strains and Dr M. Inoue for the patients' sera. This work was partly supported by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture, Japan.

REFERENCES

- DAVIES, D. A. L., MORGAN, W. T. J. & RECORD, B. R. (1955). Studies in immunochemistry. 15. The specific polysaccharide of the dominant 'O' somatic antigen of *Shigella dysenteriae*. *Biochemical Journal* **60**, 290–303.
- KAWAOKA, Y., NAIKI, M. & YANAGAWA, R. (1979). Radioimmunoassay system using a serovar-specific lipopolysaccharide antigen of *Leptospira*. *Journal of Clinical Microbiology* **10**, 313–316.
- KAWAOKA, K., OTSUKI, K. & TSUBOKURA, M. (1983). Growth temperature-dependent variation in the bacteriophage-inactivating capacity and antigenicity of *Yersinia enterocolitica* lipopolysaccharide. *Journal of General Microbiology* **129**, 2739–2747.
- LINDBERG, A. A. & HELLERQVIST, C. (1980). Rough mutants of *Salmonella typhimurium*: immunochemical and structural analysis of lipopolysaccharides from *rfaH* mutants. *Journal of General Microbiology* **116**, 25–32.
- MOLLARET, H. H. & NICOLLE, P. (1965). Sur la fréquence de la lysogenie dans l'espèce nouvelle *Yersinia enterocolitica*. *Comptes rendus hebdomadaires des séances de l'Académie des sciences* **260**, 1027–1029.
- OKAMOTO, K., INOUE, T., ICHIKAWA, H., KAMAMOTO, Y., HARA, S. & MIYAMA, A. (1980). Adherence of *Yersinia enterocolitica* to mammalian epithelial cell lines. *Microbiology and Immunology* **24**, 1013–1022.
- PAI, C. H. & MORS, V. (1978). Production of enterotoxin by *Yersinia enterocolitica*. *Infection and Immunity* **19**, 908–911.
- SCHLEIFSTEIN, J. & COLEMAN, M. B. (1939). An identified microorganism resembling *B. ligieresi* and *Pasteurella pseudotuberculosis*, pathogenic for man. *New York State Journal of Medicine* **39**, 1749–1753.
- TSUBOKURA, M., OTSUKI, K., SHIMOHARA, I. & YAMAMOTO, H. (1979). Production of indirect hemolysin by *Yersinia enterocolitica* and its properties. *Infection and Immunity* **25**, 937–942.
- WESTPHAL, O. & JANN, K. (1965). Bacterial lipopolysaccharide extraction with phenol-water and further application of the procedure. *Methods in Carbohydrate Chemistry* **5**, 83–91.